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Effect of cannabis in the intrastriatal lipopolysaccharide model of Parkinson's disease in the rat

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ABSTRACT
Cannabis is used by Parkinson's disease (PD) patients. Therefore, the present study investigated oxidative stress and neurodegeneration in brain subsequent to treatment with *Cannabis sativa* extract. The rat model of PD induced by intrastriatal injection of lipopolysaccharide (LPS) was used for this purpose. LPS or vehicle was injected into the striatum and rats were then treated daily for 15 days with cannabis extract at doses of 5, 20, 20 or 40 mg/kg (expressed as delta-9-tetrahydrocannabinol). Biochemical markers of oxidative stress and tumour necrosis factor-α (TNF-α) were determined besides brain histopathology and caspase-3 expression. Results showed that intrastriatal LPS increased oxidative/nitrosative stress indicated by increased malondialdehyde (MDA) and nitric oxide combined with decreased reduced glutathione (GSH) and paroxonase-1 (PON-1) activity in different brain regions. There were also decreased striatal levels of glutathione peroxidase (GPx) and increased TNF-α. LPS caused neuronal necrosis and apoptosis and intense caspase-3 immunostaining. Cannabis treatment decreased the elevated MDA and nitric oxide levels while increasing GSH and PON-1 activity in different brain regions. Cannabis also increased GPx activity and markedly reduced TNF-α in the striatum. Cannabis, however, did not protect against neuronal damage caused by LPS. These findings do not suggest a benefit from using cannabis in PD.

Keywords: Cannabis, lipopolysaccharide, oxidative stress, neuroinflammation, apoptosis, caspase-3

1. INTRODUCTION
Parkinson's disease (PD) is a hypokinetic neurodegenerative disorder caused by the progressive and selective death of the dopamine synthesizing cells of the mid brain substania nigra pars compacta (Hughes et al., 1992). Parkinson's disease is common in the elderly subjects being the second most common after Alzheimer's disease (Benninger et al., 2009). The cardinal features are those of slowness and difficulty in the initiation of voluntary movements, tremor at rest, muscular rigidity and postural abnormalities (Fahn, 2003). These symptoms are consequent

to the depletion of dopamine in the substantia nigra and striatum (Bernheimer et al., 1973; Hughes et al., 1992). The cause of PD is unknown in 95% of cases i.e., idiopathic PD (Pankratz and Foroud, 2007) where it is largely accepted that exposure to an environmental toxin may be the initiating event in those with genetic susceptibility (Wirdefeldt et al., 2011). In this context, a number of epidemiological studies have implicated being exposed to pesticides with the increase in the risk for the development of PD (Priyadarshi et al., 2000; Hancock et al., 2008). Increasingly accumulating evidence from both clinical and experimental studies indicated that the presence of high levels of oxidative stress and neuroinflammatory events are the major cellular pathways leading to dopaminergic cell death in PD (Drechsel and Patel, 2008; Miller et al., 2009).

Products derived from the *Cannabis sativa* plant are the most commonly abused illicit substances around the world. The most common products are marijuana from the flowering tops and leaves of the plant and the compressed resin or hashish (World Drug Report, 2020). The cannabis plant contains a large number of terpenophenolic compounds unique to the plant known as "cannabinoids" (Elsohly et al., 2017). Among these, delta-9-tetrahydrocannabinol (THC) is the most abundant cannabinoid and the one that mediates the psychoactive effects of smoked cannabis (Ashton, 2001). Other cannabinoids eg, cannabidiol, cannabigerol, and cannabichromene are present in much smaller amounts and are devoid of psychotropic properties (Elsohly et al., 2017).

In the last years, interest has been growing in the use of cannabis for a number of disease aliments eg, multiple sclerosis (Meuth et al., 2015), fibromyalgia (Fiz et al., 2011), neuropathic pain (Ellis et al., 2009) and epilepsy (Reddy and Golub, 2016; Abdel-Salam, 2021). Cannabis has also been suggested as a potential therapy for PD subjects. In the study by Venderová et al., (2004), PD subjects reported substantial improvements their symptoms when consuming a half teaspoon of fresh or dried leaves orally. Cannabinoids act on two types of G-protein-coupled receptors CBR1 and CBR2 (Pertwee and Ross, 2002). CBR1 is highly expressed in cerebral cortex, cerebellum, hippocampus and basal ganglia. Moreover, endogenous ligands of CBR1 such as anandamide and and 2-arachidonoylglycerol are found in basal ganglia which suggests that CBR1 may be involved in control of motor activity (Di-Marzo et al., 2000; Gerdeman and Fernández-Ruiz, 2008). In light of the above, the present study was designed to examine the effect of cannabis extract rich in THC on oxidative stress and neurodegeneration in brain, the key pathways underlying dopaminergic cell death in PD, using rat model of PD induced by intrastriatal injection of LPS.

2. MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats, weighing 160–170 g was included in this study. Rats were obtained from the animal house colony of the National Research Centre. Animals were kept in standardized housing with 24-27 °C ambient temperature, 60% humidity and 12-hour cycles of light and dark. Rats had unrestricted access to water and regular laboratory pellets. The experimental procedures were performed in compliance with the institutional Ethics Committee and with the Guide for Care and Use of Laboratory Animals of US National Institutes of Health (Publication No. 85-23, revised 1996).

Surgery

Rats were anaesthetized with intraperitoneal sodium pentobarbital (40 mg/kg). The hair was shaved from the fronto-occipital area and 2% iodine solution was applied for antisepsis. Using orthodontic roof motor and number 2 drills, a hole of 0.5 cm was made to the right of the bregma till the dura matter was exposed. With the use of Hamilton syringe fitted with a 30-gauge needle, LPS was injected into the right striatum at the dose of 5μg/rat (3μl/rat). A group of rats (n = 6) have had surgery but received saline injection and served as negative control. After the injection, the burr hole was sealed with bone wax and sprayed with an antibiotic.

Drugs and chemicals

Cannabis sativa plant was kindly provided by the Ministry of Justice (Egypt). Other reagents and chemicals were purchased from Sigma (St. Louis, USA).

Preparation of cannabis extract

Cannabis sativa extract was prepared from the plant's flowering tops and leaves after drying. The extraction method was that described by Turner and Mahlberg, (1984) with modification. Briefly, a mortar and pestle were used to grind 10 g of the dried plant material. The plant material was decarboxylated by putting it in a glass test tube, wrapping it in aluminium foil and heating it up in an oven to 100 °C for two hours. Afterwards, 10 ml of analytical-grade chloroform was added and the mixture was allowed to react for the night. Three extractions of the cannabis material were made. The combined fractions were then separated by filter paper and collected. The filtrate was allowed to evaporate using a mild nitrogen stream over ice that was shielded from light. The residue was

kept in a glass flask covered in aluminium and kept at 4 °C away from light. The residue was re-suspended in 2 ml of 96% ethanol for the tests and the total volume was increased to 100 ml by adding saline. Using gas chromatography-mass spectrometry, the THC level of the cannabis extract was measured and determined to be 10%.

Experimental design

Different groups of rats (n = 6/group) were used in the study. The treated groups of rats were randomly assigned as follows:

Group 1 was treated with the vehicle (intrastriatal saline) and served as negative control.

Group 2 received intrastriatal LPS (5 μg/rat) and served as positive control.

Groups 3, 4, 5 and 6 received intrastriatal LPS and were treated with cannabis extract (5, 10, 20 and 40 mg/kg which are equivalent to the active ingredient THC). Treatments were administered via the SC route daily for 15 days starting on the day of surgical treatment and LPS injection.

Determination of lipid peroxidation

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in brain supernatants using the method of Ruiz-Larrea et al., (1994).

Determination of nitric oxide

Nitric oxide was measured spectrophotometrically in supernatants as nitrite by the use of Griess reagent according to the method of Moshage et al., (1995).

Determination of reduced glutathione

The sulfhydryl group content in supernatants was determined by the 2.2-dithiobisnitrobenzoic acid assay as described by Ellman, (1959).

Determination of glutathione peroxidase activity

The activity of GPx was determined in supernatants of homogenized striata samples spectrophotometrically at 340 nm by the analysis of NADPH oxidation according to Paglia and Valentine, (1967) using glutathione peroxidase kit from Bio diagnostics (Egypt). One unit of GPx activity is defined as the amount of protein that oxidized 1mM NADPH per minute. The activity of GPx is expressed as mU/mL.

Determination of tumour necrosis factor-alpha

TNF-α was determined in supernatants by enzyme-linked immunosorbent assay using TNF-α kit (Biosource International, USA) according to the instructions provided by the manufacture.

Determination of paraoxonase-1 activity

Arylesterase activity of paraoxonase was measured spectrophotometrically in supernatants with the use of phenyl acetate as the substrate. In this assay, arylesterase/paraoxonase catalyzes the cleavage of phenyl acetate resulting in phenol formation. The rate of formation of phenol is measured by monitoring the increase in absorbance at 270 nm at 25◎C. In this assay, the cleavage of phenyl acetate into phenol is catalyzed by arylesterase/paraoxonase. Monitoring the rise in absorbance at 270 nm and 25◎C is used to determine the rate of phenol synthesis. The working reagent consisted of 20 mMTris/HCl buffer (pH 8.0) containing 1 mM calcium chloride and 4 mM phenyl acetate as the substrate. Samples diluted 1:3 in buffer are added and the change in absorbance is recorded following a 20 s lag time. Absorbance at 270 nm was taken every 15 s for 120 s using a UV-Vis Recording Spectrophotometer (Shimadzu Corporation). One unit of arylesterase activity is equal to 1 μM of phenol formed per minute. The activity is expressed in kU/l, based on the extinction coefficient of phenol of 1310 M/cm at 270 nm, pH 8.0 and 25◎C. Blank samples containing water are used to correct for the spontaneous hydrolysis of phenyl acetate (Haagen and Brock, 1992).

Brain histopathology

The representative brain samples were immediately removed and washed in saline solution. Samples were fixed in a solution of 10% phosphate buffered formalin. Following an overnight fixation, slices (3–4 mm) of brain tissue were dried in alcohol, cleaned in xylene and embedded in paraffin wax. Blocks were made and sectioned at 5 μm thickness with microtome. The tissue sections were

stained using hematoxylin and eosin and observed for histopathological changes and microphotographs were taken using microscope system (Olympus, Japan).

Immunohistochemistry for caspase-3

Immunohistochemical staining of anti-caspase-3 antibodies was performed by streptoavidin–biotin. Deparaffinized four-micrometer-thick slices were then incubated for 30 minutes at room temperature with fresh 0.3% hydrogen peroxide in methanol. The anti-caspase-3 antibody, which served as the primer antibody, was then incubated with the samples at a dilution of 1:100. H & E was used as a counterstain on the specimens. Every primary antibody was replaced with regular mouse serum to create the negative controls.

Statistical analysis

Data are presented as the mean ± SE. Data were analyzed by one-way analysis of variance. Duncan's multiple range test was used for post hoc comparison of group means using SPSS software. Effects with a probability of $p < 0.05$ were considered to be significant.

3. RESULTS

Biochemical results

Lipid peroxidation

Intra-striatal injection of LPS at 5μg/rat resulted in increased brain oxidative stress in the striatum and in other brain regions. Lipid peroxidation, as assessed by MDA levels, significantly increased in cerebral cortex (189%), midbrain (210.5%), striatum (167.2%), hippocampus (188.7%) and cerebellum (125.5%) after LPS injection. Following the administration of cannabis extract at doses of 5, 10, 20 or 40 mg/kg, a significant decrease in MDA was found in the cortex (by 37.7, 49, 54.5 and 42.2%), midbrain (by 21.6, 33.9, 53.3 and 32.3%), striatum (by 16.2, 55.1, 67.5 and 56%), hippocampus (by 16.9, 36.8, 51.1 and 23%) and cerebellum (by 24, 34.1, 49.2 and 33.7%). Cannabis given at 40 mg/kg significantly induced less decrease in MDA compared with 20 mg/kg cannabis (Figure 1) (Table 1).

Table 1 Malondialdehyde (MDA) in brain of rats given intrastriatal LPS and the effect of treatment with cannabis extract

	Saline	LPS	LPS + Cannabis (5 mg/kg)	LPS + Cannabis (10 mg/kg)	LPS + Cannabis (20 mg/kg)	LPS + Cannabis (40 mg/kg)
MDA (ng/g tissue)						
Cerebral cortex	20.0±0.76	57.8±1.8*	36.0±0.83^{*+}	29.75±0.1^{*+}	26.32±0.4^{*+}	33.43±1.4^{*+}
Midbrain	18.26±0.59	56.70±2.2*	44.47±1.1*	37.51±3.0^{*+}	25.98 ±0.5^{*+}	38.44±1.7^{*+}
Striatum	22.89±0.47	61.22±0.37*	51.33±1.2*	27.51±1.26^{+}	19.90±0.47^{+}	26.99±0.7^{+}
Hippocampus	19.36±0.70	55.96±1.30*	46.50±1.8*	35.44±1.1^{*+}	27.38±0.4^{*+}	43.10±0.9^{*+}
Cerebellum	21.16±0.89	47.81±0.64*	36.25±2.2*	31.54±1.6^{*+}	24.26±1.2^{*+}	31.69±1.5^{*+}

Data are presented as the mean ± SE. n = 6 in each group. Statistical analysis was performed using one-way ANOVA and Duncan's multiple range test was used for comparing different groups. *$p < 0.05$ as compared to saline group, +$p < 0.05$ as compared to LPS control group.

Nitric oxide

Injection of LPS produced a significant increase in nitric oxide in the cortex (by 553.7%), midbrain (by 536.4%), striatum (by 617.7%), hippocampus (by 331.5%) and cerebellum (by 358.4%). Cannabis caused a significant reduction in the elevated nitric oxide levels with the effect being most marked at 20 mg/kg of cannabis. Thus, nitric oxide was reduced in cortex (by 31.7, 60.4, 73.1 and 55.9%), midbrain (by 44.9, 46.7, 74.1 and 70.2%), striatum (by 17.9, 29, 62.7 and 30.3%), hippocampus (by 15.4, 54.3, 58.3 and 17.6%), and cerebellum (by 14.5, 36, 63.8 and 17.2%) by different doses of the extract (Figure 2) (Table 2).

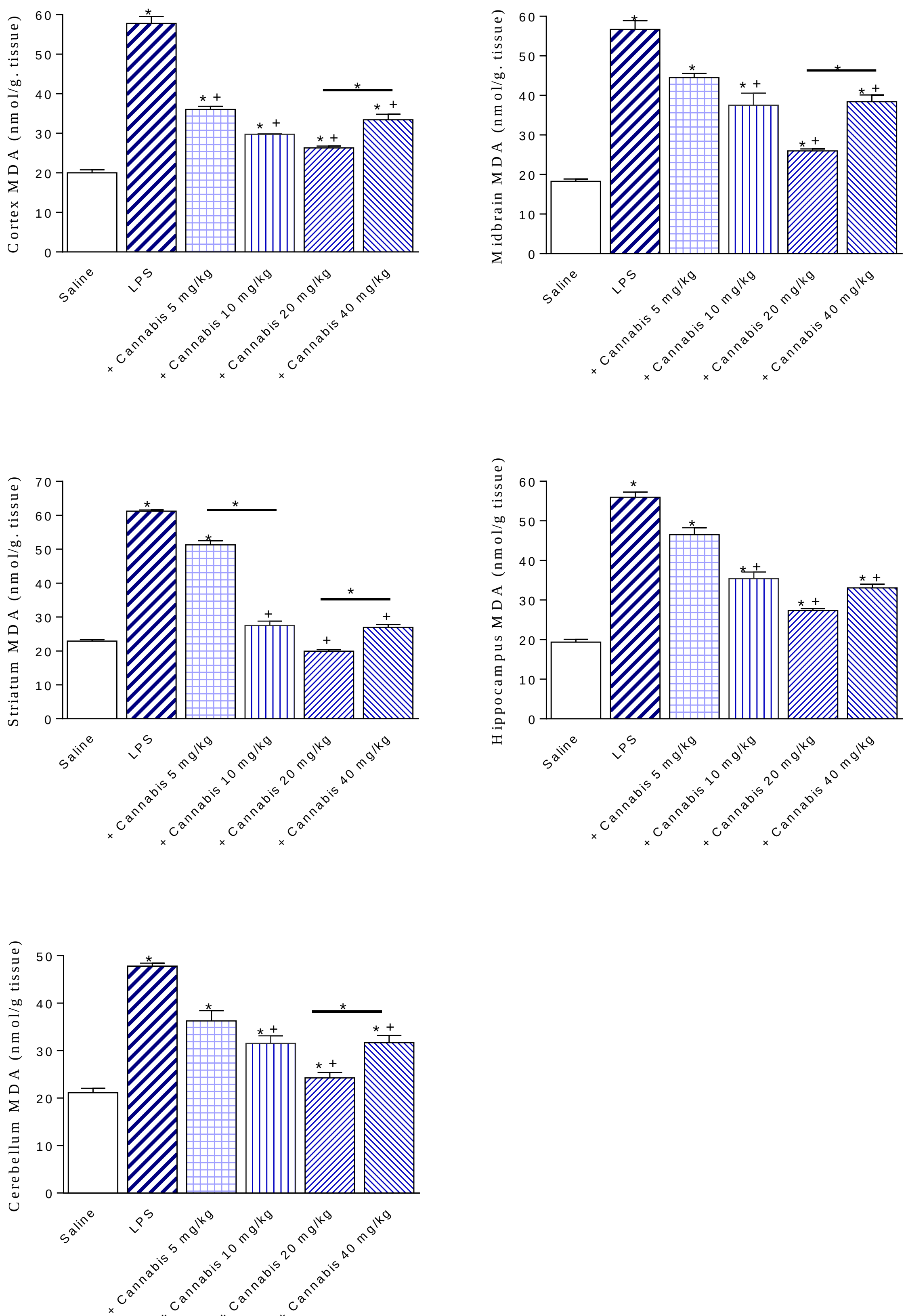

Figure 1 Malondialdehyde (MDA) in brain of rats after intrastriatal lipopolysaccharide (LPS) injection and the effect of cannabis. *p < 0.05 as compared to saline group and between various groups, as demonstrated by the graph, +p < 0.05 as compared to LPS control group

Table 2 Nitric oxide levels in brain of rats given intrastriatal LPS and the effect of treatment with cannabis extract

	Saline	LPS	LPS + Cannabis (5 mg/kg)	LPS + Cannabis (10 mg/kg)	LPS + Cannabis (20 mg/kg)	LPS + Cannabis (40 mg/kg)
Nitric oxide (μmol/g tissue)						
Cerebral cortex	28.2 ± 2.0	178.67±10.3*	122.0±8.1*+	70.67±2.50*+	48.0±3.3*+	50.8±4.0*+
Midbrain	28.6±1.3	182.0±5.7*	120.3±6.0*+	96.93 ±6.8*+	47.2±3.56*+	54.3±2.1*+
Striatum	27.6±1.43	198.1±7.6*	162.67±8.9*	140.6±10.0*+	73.84±4.4*+	79.0±6.5*+
Hippocampus	27.0±1.63	116.48±9.1*	98.53±4.7*	53.2±3.0*+	48.56±1.9*+	47.0±3.4*+
Cerebellum	29.3±1.7	134.27±6.4*	114.8±6.7*	86.0±4.1*+	48.56±2.5*+	46.2±4.7*+

Data are presented as the mean ± SE. n = 6 in each group. Statistical analysis was performed using one-way ANOVA and Duncan's multiple range test was used for comparing different groups. *$p < 0.05$ as compared to saline group, +$p < 0.05$ as compared to LPS control group.

Reduced glutathione

Compared to saline controls, the reduced glutathione (GSH) concentrations in different brain regions significantly decreased in LPS injected rats. GSH decreased in the cortex (by 37.3%), midbrain (by 30.5%), striatum (by 40.8%), hippocampus (by 31.7%) and cerebellum (by 33.8%) with respect to saline control levels. A dose-dependent increase in GSH was observed in brain of rats treated with cannabis. Significant increase in GSH was found in the cortex (by 31.1, 43.9 and 40.3%), striatum (28.0, 31.5 and 33.1%), midbrain (by 22.6, 42.8 and 29%) and cerebellum (by 27.6, 41.5 and 34%) of rats receiving cannabis doses of 10, 20 and 40 mg/kg, respectively. Reduced glutathione increased in the hippocampus by 14.6 and 34.2% by 20 and 40 mg/kg of cannabis, respectively (Figure 3) (Table 3).

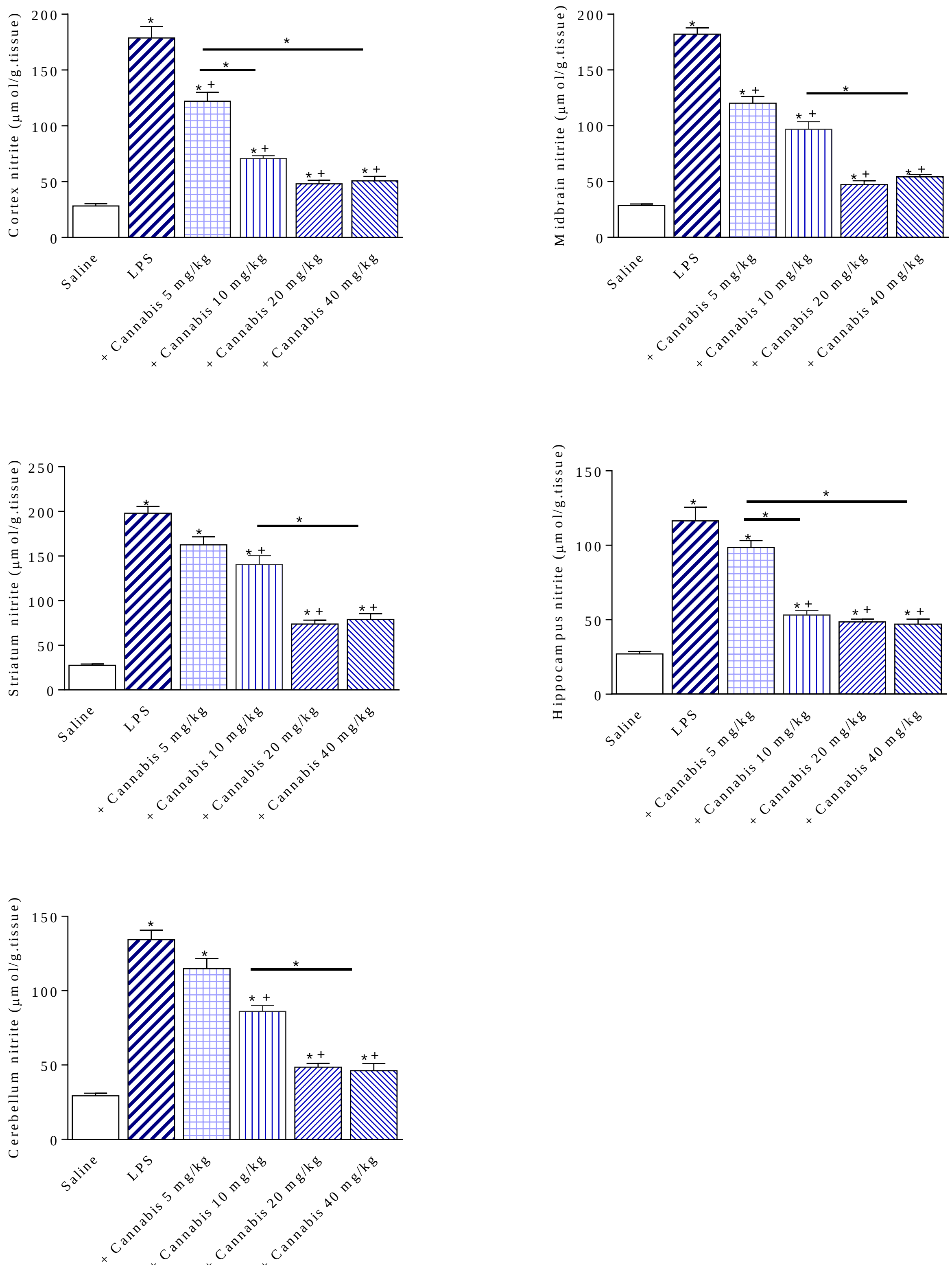

Figure 2 The increase in nitric oxide in rat brain after intrastriatal lipopolysaccharide (LPS) injection and the effect of cannabis. *$p < 0.05$ as compared to saline group and between various groups, as demonstrated by the graph, +$p < 0.05$ as compared to LPS control group

Table 3 Reduced glutathione (GSH) levels in brain of rats given intrastriatal LPS and the effect of treatment with cannabis extract

	Saline	LPS	LPS + Cannabis (5 mg/kg)	LPS + Cannabis (10 mg/kg)	LPS + Cannabis (20 mg/kg)	LPS + Cannabis (40 mg/kg)
GSH (μmol/ g tissue)						
Cerebral cortex	4.13±0.02	2.73±0.04*	2.94±0.06*	3.58±0.05+	3.93±0.02+	3.83±0.05+
Midbrain	4.11±0.08	2.87±0.06*	3.30±0.02*	3.52±0.02*+	3.7±0.034+	4.10±0.02+
Striatum	4.34±0.12	2.57±0.06*	3.29±0.08*+	3.38±0.06*+	3.78±0.05*+	3.42±0.04*+
Hippocampus	4.32±0.05	2.95±0.05*	3.24±0.07*	3.29±0.08*	3.38±0.02*	3.96±0.04+
Cerebellum	4.26±0.05	2.82±0.09*	2.97±0.02*	3.60±0.10*+	3.99±0.07+	3.78±0.03+

Data presented are the mean ± SE. n = 6 in each group. Statistical analysis was performed using one-way ANOVA and Duncan's multiple range test. *p < 0.05 as compared to saline group, +p < 0.05 as compared to LPS control group.

Paraoxonase 1

Lipopolysaccharide led to significant and marked decrease of PON1 activity in the cortex (by 71.6%), striatum (by 86%), midbrain (by 82.3%), hippocampus (by 84.9%) and cerebellum (by 74.5%). In LPS-treated rats, cannabis resulted in increased PON1 activity in a dose-dependent manner (Figure 3). Significant increase in PON1 activity was recorded in cortex (by 126.5, 156.3 and 239.9%), midbrain (by 64.6, 96.5 and 203.1%), hippocampus (by 69.3, 215.7 and 249%) and cerebellum (by 82.8, 186.7 and 266.3%) after receiving cannabis doses of 10, 20 and 40 mg/kg, respectively. PON1 activity increased in the striatum by 74.5, 109.1, 215.7 and 249% after treatment with different doses of cannabis (5, 10, 20 and 40 mg/kg), respectively (Figure 4) (Table 4).

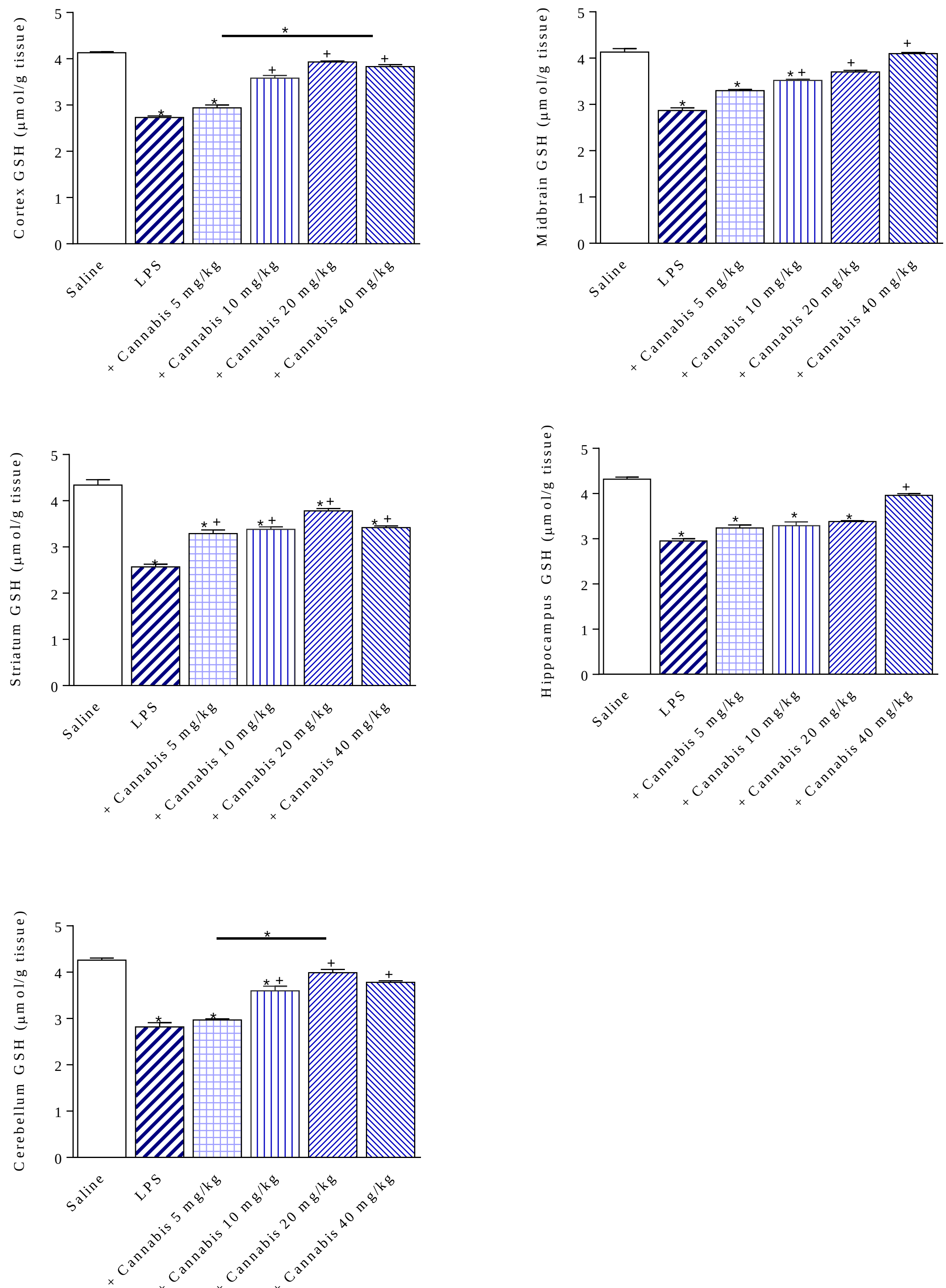

Figure 3 Reduced glutathione (GSH) in the rat brain after intrastriatal lipopolysaccharide (LPS) injection and the effect of cannabis. *$p < 0.05$ as compared to saline group and between various groups, as demonstrated by the graph, +$p < 0.05$ as compared to LPS control group

Table 4 Paroxonase-1 activity (PON-1) in brain of rats given intrastriatal LPS and the effect of treatment with cannabis extract.

	Saline	LPS	LPS + Cannabis (5 mg/kg)	LPS + Cannabis (10 mg/kg)	LPS + Cannabis (20 mg/kg)	LPS + Cannabis (40 mg/kg)
PON-1 (kU/l)						
Cerebral cortex	12.10±0.23	3.43±0.27*	3.11±0.17*	7.77±0.31*+	8.79±0.26*+	11.66±0.42+
Midbrain	10.94±0.83	2.79±0.12*	2.90±0.11*	5.09±0.32*+	8.01±0.30*+	10.22±0.26*+
Striatum	12.950.61	2.29±0.12*	2.43±0.31*+	3.77±0.20*+	4.50±0.16*+	6.94 ±0.36*+
Hippocampus	10.17 ± 31	1.53±0.11*	1.70 ±0.16*	2.59±0.1*+	4.83±0.15*+	5.34 ± 0.21*+
Cerebellum	10.94±0.76	1.53±0.14*	2.67±0.34*	3.20±0.20*+	4.83±0.18*+	5.340±0.40+

Data presented are the mean ± SE. n = 6 in each group. Statistical analysis was performed using one-way ANOVA and Duncan's multiple range test. *$p < 0.05$ as compared to saline group, +$p < 0.05$ as compared to LPS control group.

Gluathione peroxidase

In striatum of LPS-injected rats, GPx activity significantly decreased by 33.3% with respect to saline control levels. Rats treated with cannabis at doses of 5, 10, 20 or 40 mg/kg showed increased GPx activity by 18.3, 19.9, 34.9 and 94.9%, respectively (Figure 5A) (Table 5).

Tumour necrosis factor-α

Lipopolysaccharide injection into the striatum led to robust increase in striatal TNF-α by 608.2% (283.3 ± 1.8 vs. saline control value of 40.0 ± 0.76 pg/ml, $p < 0.05$). Striatal TNF-α significantly decreased following different doses of cannabis by 64.7, 70.6, 79.7 and 78.8% with respect to LPS control levels (Figure 5B) (Table 5).

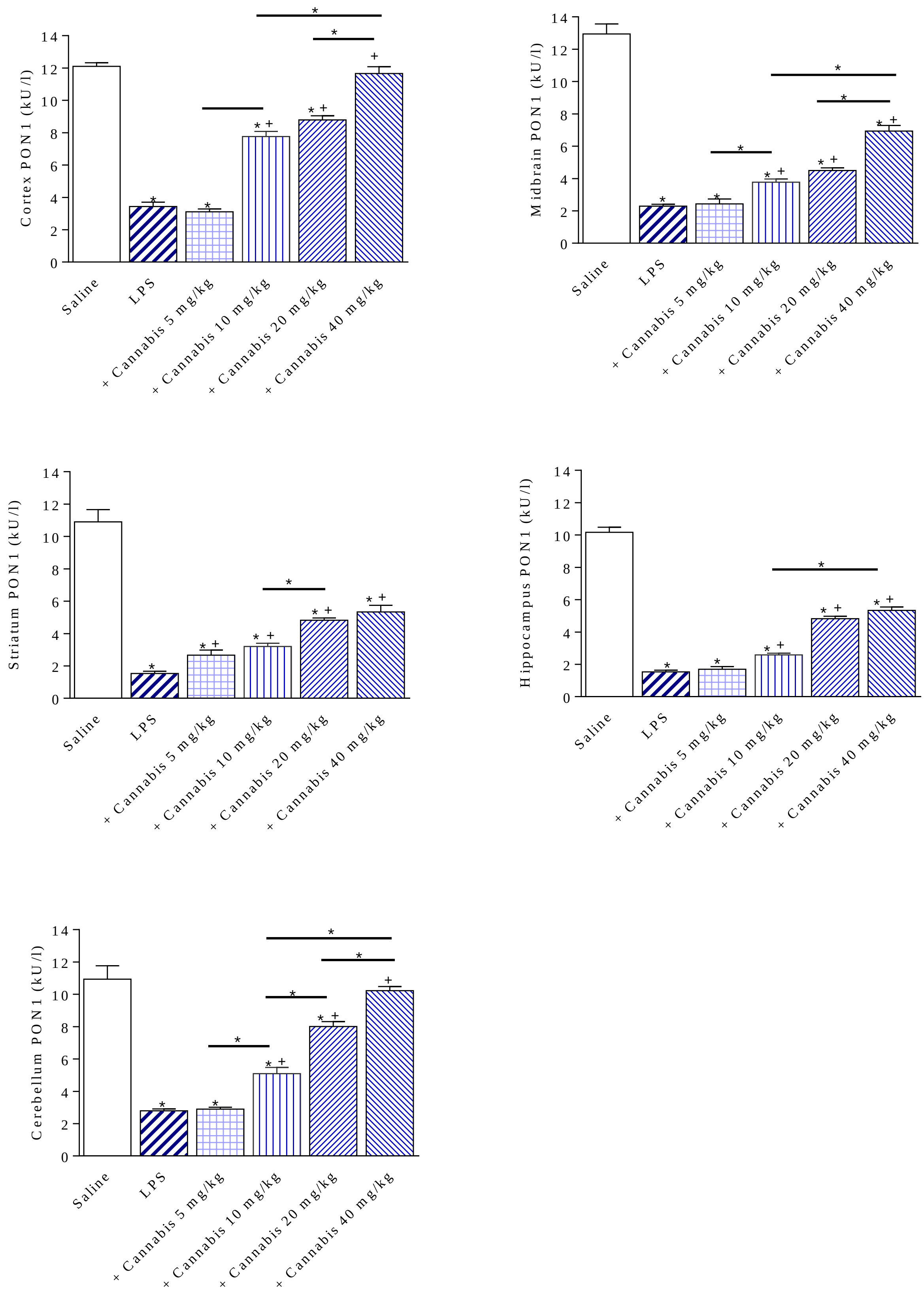

Figure 4 Paraoxonase-1 (PON-1) activity in brain of rats after intrastriatal lipopolysaccharide injection and the effect of cannabis. *p < 0.05 as compared to saline group and between various groups, as demonstrated by the graph, +p < 0.05 as compared to LPS control group

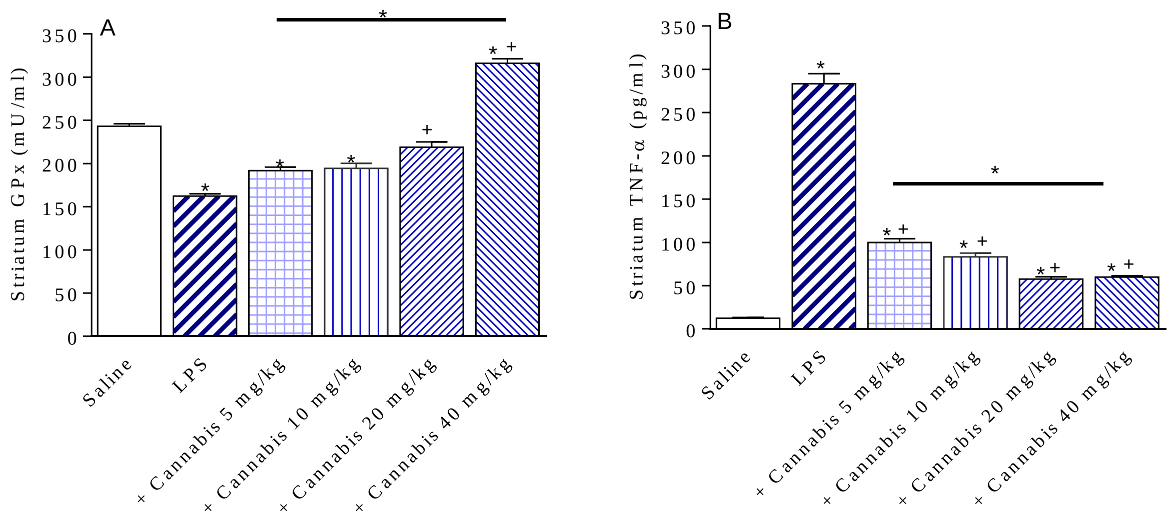

Figure 5 (A) Glutathione peroxidase (GPx) activity and (B) Tumour necrosis factor-α (TNF-α) in striatum of rats after intrastriatal lipopolysaccharide injection (LPS) and the effect of different treatments. *p < 0.05 as compared to saline group and between various groups, as demonstrated by the graph, +p < 0.05 as compared to LPS control group

Table 5 The activity of glutathione peroxidase (GPx) and tumour necrosis factor-alpha (TNF-α) levels in the striatum of rats given intrastriatal LPS and the effect of treatment with cannabis extract

	Saline	LPS	LPS + Cannabis (5 mg/kg)	LPS + Cannabis (10 mg/kg)	LPS + Cannabis (20 mg/kg)	LPS + Cannabis (40 mg/kg)
GPx (mU/ml)	243.15±2.79	162.2±2.86*	191.81±4.06*	194.50±5.7*	218.8±6.3+	316.1±5.2*+
TNF-α (pg/ml)	12.5±0.71	283.3±11.8*	100.0±4.30*+	83.3±4.4*+	57.5±2.8*+	60.0±1.4*+

Data presented are the mean ± SE. n = 6 in each group. Statistical analysis was performed using one-way ANOVA and Duncan's multiple range test. *p < 0.05 as compared to saline group, +p < 0.05 as compared to LPS control group.

Histopathological results

Cerebral cortex

The cerebral cortex sections from rats in the saline control group exhibited the normal histological appearance (Figure 6A). In the LPS only-treated rats, there were neuronal cell degeneration, necrosis and foamy appearance of cytoplasm, pyknotic cells, apoptotic cells and slight vacuolation of neuropil. In addition, perivascular edema and dilatation of the vessels were seen (Figure 6B). Rats treated with cannabis at doses of 5 & 10 mg/kg after LPS injection showed mild cell damage and shrinkage of neurons (Figure 6C, D). Whereas, rats treated with cannabis at doses of 20 & 40 mg showed shrinkage of neurons, cell damage and basophilic neurons with pyknosis (Figure 6E, F).

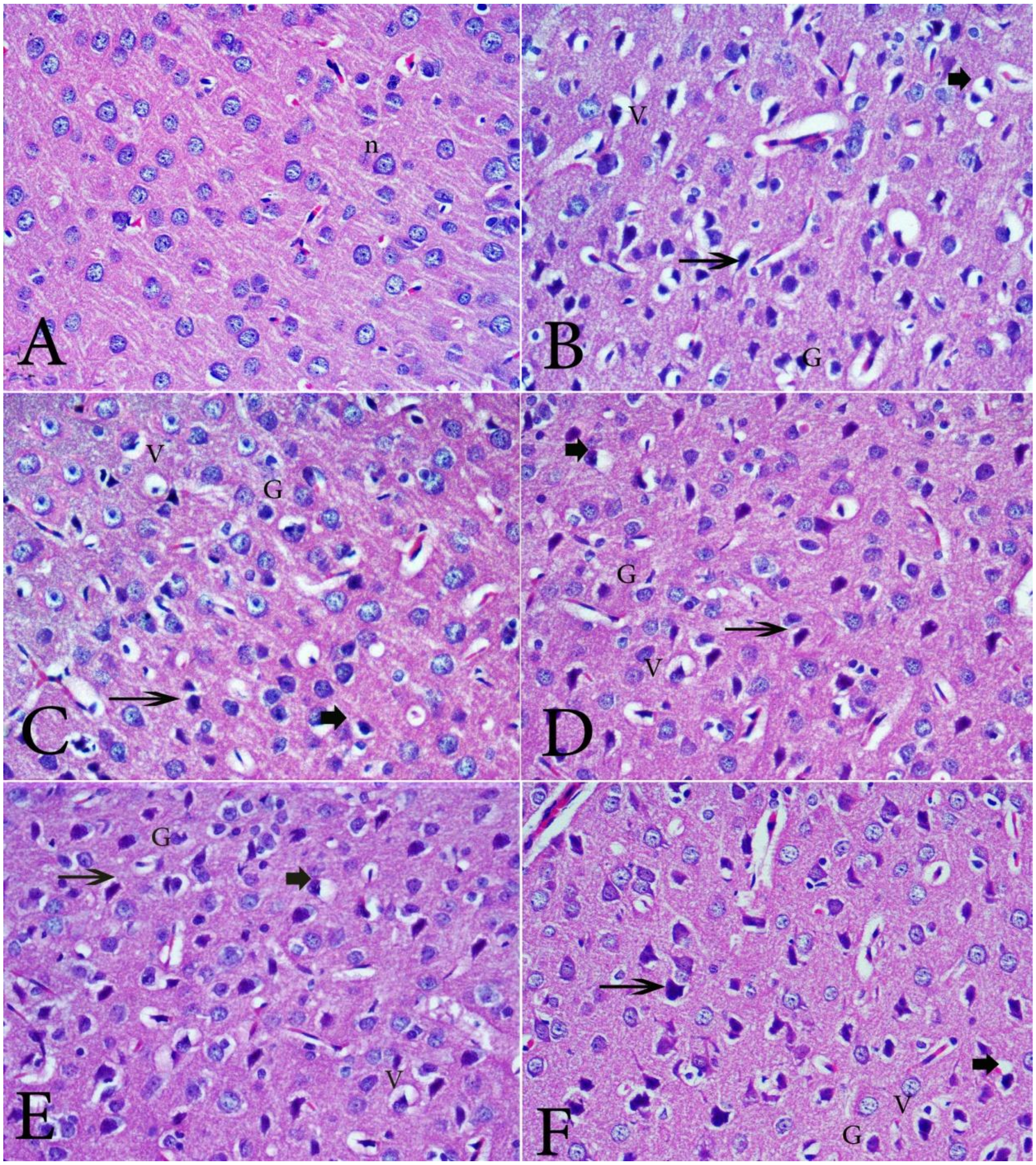

Figure 6 Photomicrographs of the cerebral cortex from rats treated with: (A) Saline. (B) LPS: Neuronal cell degeneration, pyknotic nucleus of neuron (arrow) and glia cells (G) with apoptotic cells (arrowhead), slight vacuolation of neuropil (V). (C) LPS+ cannabis 5 mg/kg: Pyknotic nucleus of neuron (arrow) and glia cells, apoptotic cells (arrowhead), and vacuolation of neuropil (V). (D) LPS+ cannabis 10 mg/kg; pyknotic nucleus of neuron (arrow) and glia cells (G) with apoptotic cells (arrowhead), slight vacuolation of neuropil (V). (E) LPS+ cannabis 20 mg/kg; cell damage and shrinkage of neurons, pyknotic nucleus of neuron (arrow) and glia cells (G), apoptotic cells (arrowhead), slight vacuolation of neuropil (V). (F) LPS+ cannabis 40 mg/kg: Obvious cell damage and shrinkage of neurons, pyknotic nucleus of neuron (arrow) with and glia cells (G), dilated blood vessels (DV), apoptotic cells (arrowhead) and slight vacuolation of neuropil (V) (H & E X 400)

Striatum

Sections from the control group given saline showed normal histology (Figure 7A). Rats treated with LPS only showed neuronal cell degeneration, pyknotic and apoptotic cells and vacuolation of neuropil (Figure 7B). Rats treated with cannabis at doses of 5 & 10 mg/kg showed mild cell damage and shrinkage of neurons (Figure 7C, D) and those treated with cannabis at doses of 20 & 40 mg showed shrinkage of neurons (Figure 7E, F).

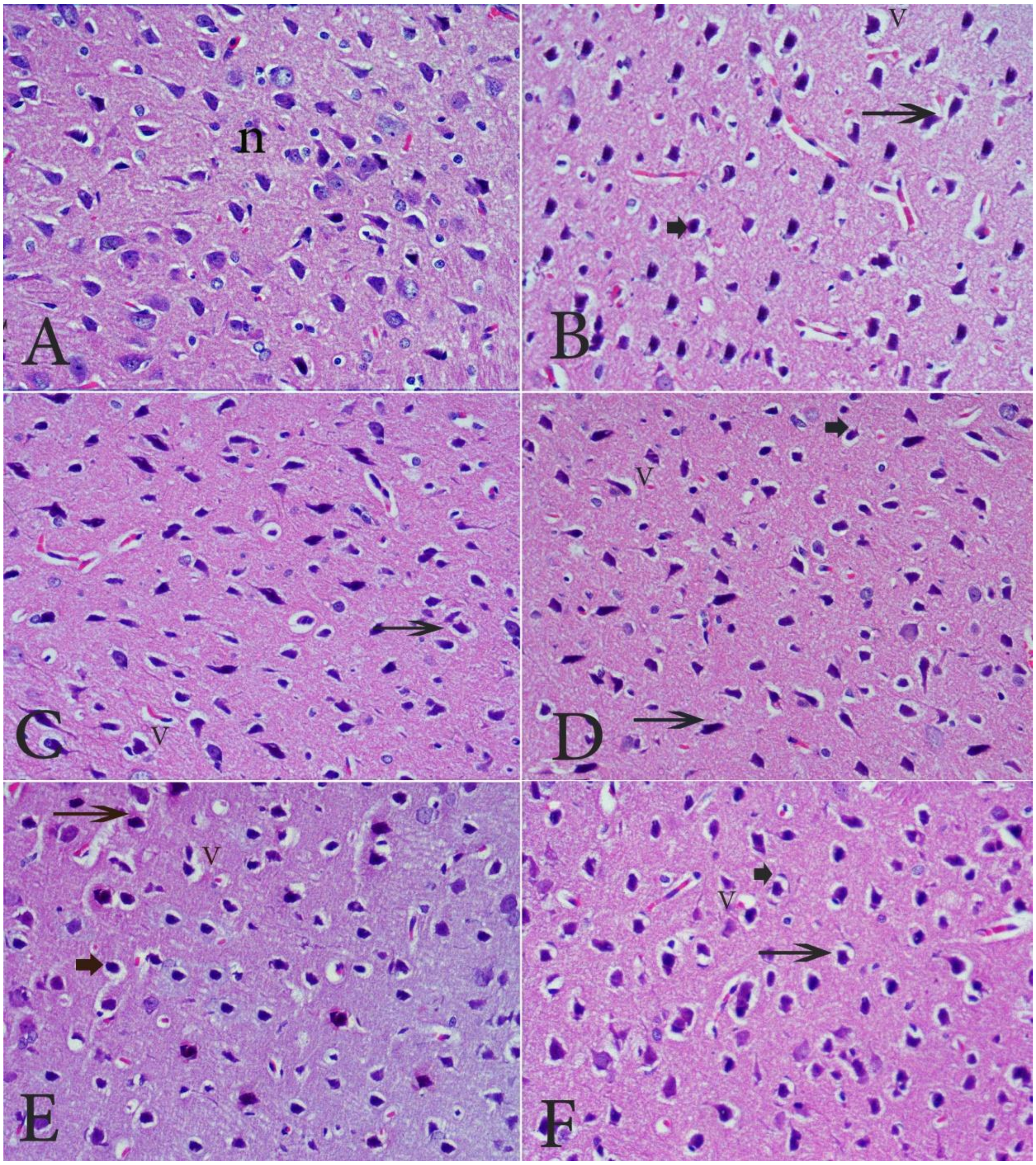

Figure 7 Photomicrographs of the striatum of rats given: (A) Saline. (B) LPS: Neuronal cell degeneration, pyknotic of nucleus (arrow) apoptotic cells (arrowhead), vacuolation of neuropil (V). (C) LPS+ cannabis 5 mg/kg: Cell damage and pyknotic of nucleus (arrow) with slight vacuolation of neuropil (V). (D) LPS+ cannabis 10 mg/kg: cell damage, pyknotic of nucleus (arrow), apoptotic cells (arrowhead) and slight vacuolation of neuropil (V). (E) LPS+ cannabis 20 mg/kg: Cell damage, pyknotic of nucleus (arrow), apoptotic cells (arrowhead) and slight vacuolation of neuropil (V). (F) LPS+ cannabis 40 mg/kg: Cell damage, pyknotic of nucleus (arrow), apoptotic cells (arrowhead) and slight vacuolation of neuropil (V) (H & E X 400)

Hippocampus

Sections of the hippocampal region from the saline controls showed normal neurons (Figure 8A). LPS caused disorganization of normal architecture and pyknosis of neuronal cells (Figure 8B). However, the hippocampus showed less damage compared with the cortex and striatum. Following treatment with cannabis, the hippocampus showed disorganization and loss of neuronal cells, some of which had pale nuclei while others were dark (Figure 8C, D, E, F).

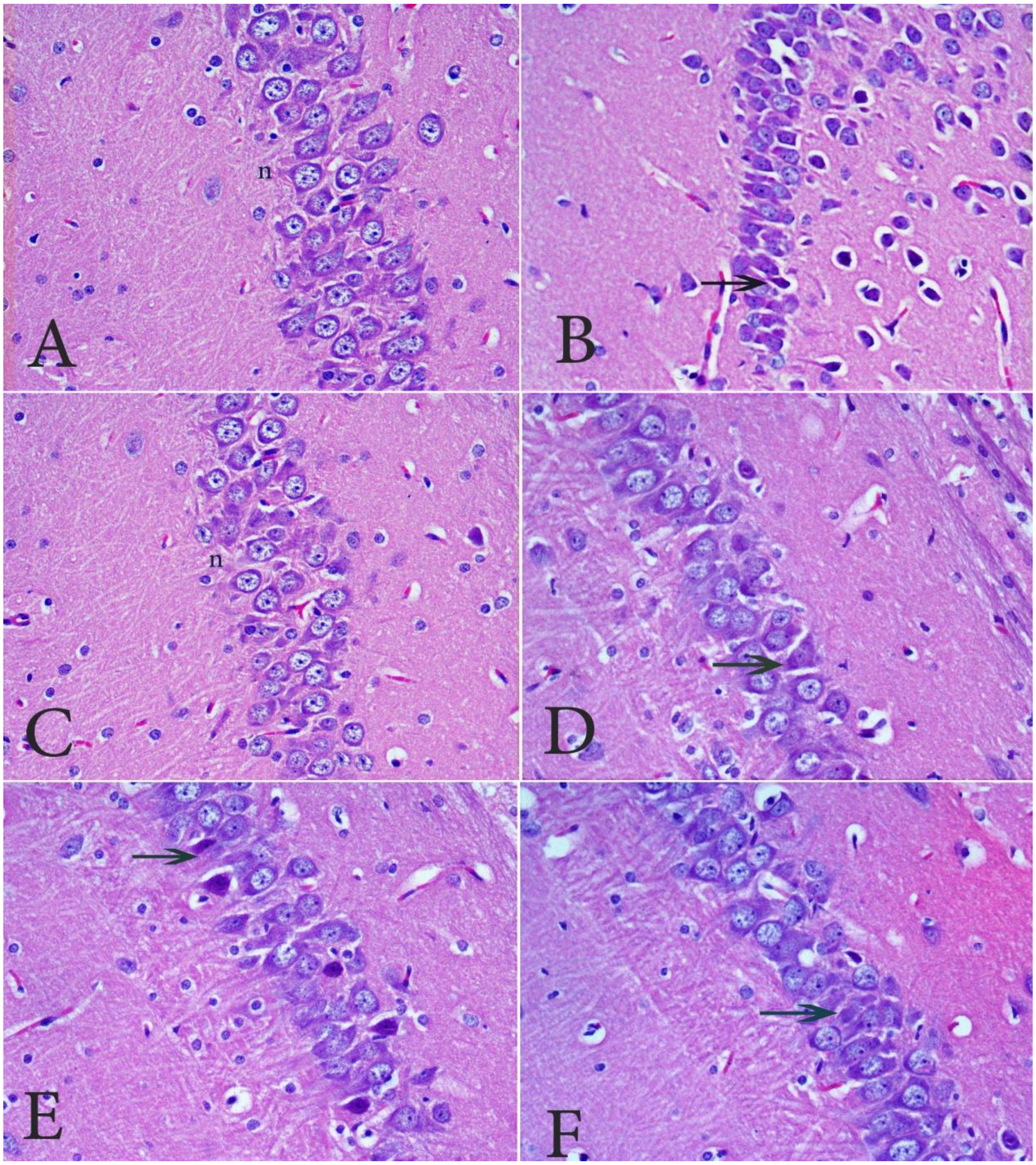

Figure 8 Photomicrographs of the hippocampus of rats after: (A) Saline: Normal neuronal cells of hippocampus. (B) LPS: Pyknotic neurons. (C) LPS+ cannabis 5 mg/kg: Pyknotic neurons. (D) LPS+ cannabis 10 mg/kg: Pyknotic neurons. (E) LPS+ cannabis 20 mg/kg: Pyknotic neurons. (F) LPS+ cannabis 40 mg/kg: Pyknotic neurons (H & E X 400)

Caspase-3 immunostaining

Immunohistochemical staining for caspase-3 showed normal distribution in the control group as a negative reaction in cortex (9A). The LPS only group showed marked increase in immunostaining, becoming dense and more widespread, indicated by brown color (Figure 9B). The results of immunohistochemical reaction in group that received LPS and cannabis (Figure 9C-E). The immunoreactivity for caspase-3 in group administered LPS and cannabis at dose of 5 & 10 mg/kg was less intense than that in the group given LPS and cannabis at doses of 20 & 40 mg/kg.

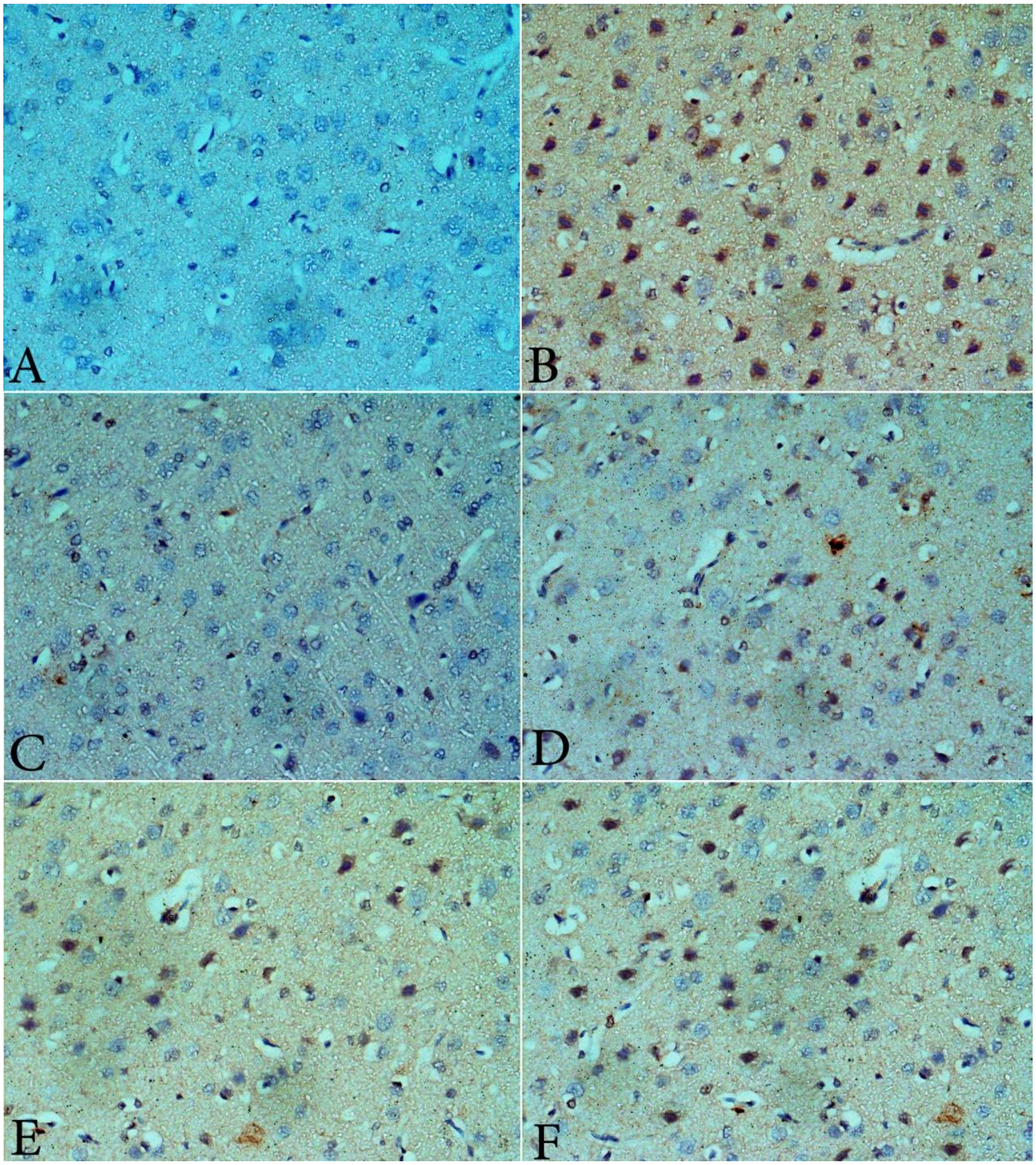

Figure 9 Caspase-3 immunohistochemistry of cortex. (A) Saline: Negative immunostaining for caspase-3. (B) LPS: Large numbers of caspase-3-immunolabelled cells were observed. (C) LPS+ cannabis 5 mg/kg: Few immunostained cells for caspase-3. (D) LPS+ cannabis 10 mg/kg: Mild immunoreactivity of caspase-3. (E) LPS+ cannabis 20 mg/kg: Mild immunoreactivity for caspase-3. (F) LPS+ cannabis 40 mg/kg: Moderate immunostaining for caspase-3 (H & E X 400)

4. DISCUSSION

This study's objective was to assess the effects of cannabis extract on oxidative stress, neuroinflammation and neurodegeneration in the model of PD induced by intrastriatal injection of LPS in the rat. Our data shows that the single intrastriatal administration of LPS (5 μg/rat) induced significant increments in lipid peroxidation evidence by increased levels of malondialdehyde, indicative of oxidative damage to polyunsaturated fatty acids. The increase in lipid peroxidation was found in the striatum, cerebral cortex, midbrain, hippocampus and cerebellum. There was also significantly increased brain content of nitric oxide. This was accompanied by marked decrease in reduced glutathione, an antioxidant and free radical scavenger and inhibition of paraoxonase-1 activity in different brain regions. Moreover, LPS evoked a pronounced increase in tumour necrosis factor-α (TNF-α) and decreased glutathione peroxidase activity (GPx) in the striatum. LPS caused marked neurodegeneration and increased caspase-3 immunoreactivities in the cerebral cortex. These findings indicate the presence of significant oxidative stress and inflammatory process in brain of LPS-injected rat which is associated with brain neurodegeneration.

The intrastriatal injection of the inflammogen LPS in rodents has been utilized by several investigators in an attempt to model idiopathic PD. These studies reported microglia activation and strong inflammatory response in brain following the injection of LPS. Choi et al., (2007) found that LPS treatment (5μg/rat) induced up regulation of iNOS, 3-nitrotyrosine, 4-hydroxynonenal and 8-hydroxy-2'-deoxyguanosine indicative of oxidative/nitrosative stress and consequent damage to the cell biomolecules e.g., lipids, proteins and DNA. Noworyta-Sokolowska et al., (2013) using intrastriatal micro dialysis probe and LPS doses up to 40 μg/rat reported increased hydroxyl (OH•) radical, increased extracellular glutamate and adenosine levels and reduced striatal dopamine content. LPS injections into the striatum induced activated microglia and astrocytes in cortex and striatum (Deng et al., 2021), infiltration of neutrophils in ipsilateral hemisphere (Rajkovic et al., 2019) and resulted in degeneration of dopaminergic neurons (Hunter et al., 2009; Deng et al., 2021).

In this study, treatment with cannabis extract was found to reduce brain oxidative stress, decreasing the elevated MDA and nitric oxide levels and increasing GSH content in different brain regions. Cannabis also increased GPx activity and markedly reduced TNF-α in the striatum of LPS-injected rats. The inhibition of lipid peroxidation, decreased brain nitric oxide and increase in reduced glutathione is consistent with previous studies in rats treated with systemic LPS (Abdel-Salam et al., 2014; Abdel-Salam et al., 2022) and intrastriatal rotenone (Abdel-Salam et al., 2015). Cannabis extracts also alleviated the increase in MDA and nitric oxide besides increasing GSH levels and catalase activity in brain of AlCl3-treated rats (Abdel-Salam et al., 2015a; Kubiliene et al., 2021). Cannabis thus appears to decrease oxidative stress evoked in brain tissue by injection of LPS.

The most prevalent non-protein thiol in a cell is glutathione which exists mostly in its reduced form (GSH). Reduced glutathione is important in maintaining the redox balance of the cell by protecting against oxidative/nitrosative stress. Glutathione functions as an antioxidant and can scavenge various reactive species e.g., hypochlorous acid, peroxynitrite, lipid peroxyl radical, peroxynitrite, and H2O2 directly and indirectly through enzymatic reactions by glutathione peroxidase (Wu et al., 2004; Martin and Teismann, 2009). Lowered mitochondrial GSH levels in dopaminergic cells has been shown to result in increased oxidative stress, reduced activity of mitochondrial complex I and decreased mitochondrial function (Jha et al., 2000).

In rat brain slices, glutathione depletion resulted in the increased carbonylation of several proteins which is likely to involve superoxide and H2O2 generation (Bizzozero et al., 2006). Other studies have shown that glutathione depletion in mesencephalic cultures-initiated phospholipase A2-dependent release of arachidonic acid and the production of superoxide radicals during arachidonic acid metabolism that can cause damage to dopaminergic cells (Mytilineou et al., 2002). Glutathione levels were decreased in the substantia nigra in PD subjects (Sian et al., 1994; Pearce et al., 1997) which implicates alterations in GSH in the disease process. Conversely, it could be suggested that provision of glutathione might help to lessen the progression of neurodegeneration by virtue of its antioxidant actions.

The gaseous molecule nitric oxide is a short-lived free radical with important physiological functions in brain e.g., neurotransmitter release, synaptic plasticity, vascular tone regulation and the immune response (Yun et al., 1997; Lundberg and Weitzberg, 2022). Excessive production of nitric oxide is, however, neurotoxic. This occurs in inflammatory conditions by the action of the inducible form of nitric oxide synthase (iNOS) induced in microglia cells in response to pro-inflammatory cytokines such as interleukin-1β, TNF-α, interferon-γ, reactive oxygen metabolites and bacterial LPS endotoxin (Moncada et al., 1991). The neurotoxic effects of nitric oxide are largely thought to be mediated by the potent oxidant peroxynitrite formed by the reaction between nitric oxide and superoxide (O2•-) (Pacher et al., 2007; Knott and Bossy-Wetzel, 2009).

The finding that treatment with cannabis extract resulted in markedly decreased concentrations of brain nitric oxide is in agreement with prior findings (Abdel-Salam et al., 2014, 2015b), suggesting that cannabis inhibits nitric oxide release. There have been reports of various cannabinoids inhibiting the formation of inducible nitric oxide. These included THC, the psychoactive constituent of cannabis (Jeon et al., 1996) and the non-psychotropic cannabinoids cannabidiol, cannabichromene (Romano et al., 2013) and cannabigerol (Borrelli et al., 2013). A study by Jeon et al., (1996) reported attenuation by THC of iNOS transcription and the production of nitric oxide by LPS-stimulated macrophages via an inhibitory effect on the activation of nuclear factor-kappa B/Rel. Other studies showed that LPS-stimulated nitric oxide production by macrophages was inhibited by cannabichromene (Romano et al., 2013) and cannabigerol (Borrelli et al., 2013). Cannabigerol reduced iNOS protein (but not mRNA) expression (Borrelli et al., 2013). CP 55,940, a synthetic cannabinoid having high affinity for CB1 and CB2 receptors (Felder et al., 1995) inhibited interferon γ/LPS-induced nitric oxide production by microglial cells, partly through the CB1 receptor (Cabral et al., 2001). It also inhibited nitric oxide release from rat cortical microglial cells (Waksman et al., 1999) and mouse astrocytes after endotoxin/cytokine stimulation (Molina-Holgado et al., 2002). Cannabinoids thus might exert neuroprotective effects by reducing the generation of nitric oxide by macrophages.

Our results also demonstrated inhibition of striatal TNF-α in brain of LPS-treated rats by cannabis. We have previously showed that administration of cannabis extract reduced the increased TNF-α in striatum of rats receiving intrastriatal rotenone (Abdel-Salam et al., 2015b) or in cerebral cortex of ketamine-treated rats (Abdel-Salam et al., 2021). TNF-α is a proinflammatory cytokine that is produced mainly by activated macrophages in response to a wide variety of infectious or inflammatory stimuli and has been implicated in pathogenesis of neurodegenerative diseases (Tweedie et al., 2007; Frankola et al., 2011). It has been demonstrated that cannabinoids alter cytokine expression in activated microglia. Thus, THC and to lesser extent anandamide reduced the amounts of LPS-induced messenger RNAs (mRNAs) for TNF-α and other proinfammatory cytokines e.g., IL-1β, IL-1α and IL-6 in rat cortical microglial cells (Puffenbarger et al., 2000). Other studies, however, reported that THC had no effect on mRNA level for TNF-α but suppressed the maturation and secretion of TNF-α from LPS-stimulated macrophages (Fischer-Stenger et al., 1993; Zheng and Specter, 1996). LPS-induced TNF-α release from rat microglia was also inhibited by anandamide and 2-arachidonylglycerol, two naturally occurring cannabinoids as well as by the synthetic cannabinoids CP 55,940, (+) WIN 55,212-2 and HU210 (Facchinetti et al., 2003).

Paraoxonase-1 is a calcium-dependent esterase and lactonase which participates in the hydrolysis of some organophosphorus insecticides, nerve agent and lipid hydroperoxides as well as in the metabolism of many other xenobiotic (Costa et al., 2008). The enzyme exerts antioxidant and antiinflammatory actions, inhibiting the release of TNF-α, IL-6 and ROS from macrophages (Aharoni et al., 2013). Deficiency of PON-1 results in increased oxidative stress in serum and macrophages (Rozenberg et al., 2003) and down-regulated a number of brain proteins important for cell defense against oxidative stress e.g., superoxide dismutase, DJ-1 and Park-7 (Suszyńska-Zajczyk et al., 2014). The enzyme is considered to have an important role in a number of neurological disorders (Menini and Gugliucci, 2014). This is particularly the case in PD because; (i) there is evidence from epidemiological studies that exposure to organophosphate insecticides increases the propensity for the development of PD (Manthripragada et al., 2010; Narayan et al., 2013); (ii) the catalytic efficiency of PON-1 has been shown to determine the susceptibility to the neurotoxicity of organophosphtes (Lee et al., 2013). Since the enzyme hydrolyzes the active metabolites of some insecticides, it could thus protect against the increased risk for the occurrence of PD in genetically susceptible individuals exposed to these compounds.

In this study, the arylesterase activity of PON-1 was shown to be markedly inhibited in different brain regions after intrastriatal LPS injection but substantially increased by treatment with cannabis extract especially in cerebral cortex and cerebellum where enzyme activity was resorted to its saline control value. In previous studies, the inhibition in PON-1 activity in brain after systemic LPS/rotenone injection in mice (Abdel-Salam et al., 2014) or following intrastriatal rotenone injection in the rat (Abdel-Salam et al., 2015b) was also alleviated by cannabis. The enzyme is inactivated by oxidants (Sozmen et al., 2008) while its activity is increased by antioxidants (Costa et al., 2005). Therefore, the increase in PON-1 activity after administering cannabis could be due to a lower level of oxidative stress.

While administering cannabis was shown in this study and in previous reports (Abdel-Salam et al., 2014, 2015a, b) to decrease oxidative stress and neuroinflammation evoked in brain tissue by a number of toxicants, it failed to provide neuroprotection. In their study, Costa and Colleoni, (2000) found that repeated administration of THC at the dose of 10 mg/kg caused a decrease in brain mitochondria oxygen consumption and uncoupled oxidative phosphorylation. In addition, a study by Volkow et al., (1996) reported that marijuana abusers exhibited a decrease in cerebellar metabolism as compared to normal subjects. Moreover, rats treated with cannabis showed marked reduction in brain glucose (Abdel-Salam et al., 2013) and decreased glucose uptake (Miederer et al., 2017). Repeated exposure to THC in mice was shown to disrupt the expression of mitochondrial proteins and impair mitochondrial respiration (Kolb et al., 2018).

In rats, repeated administration of cannabis caused significant decrease in serum level of the transcription factor, nuclear respiratory factor-2 which has important role in mitochondrial respiratory function and oxidative phosphorylation (Abdel-Salam et al., 2020). These observations suggest that cannabis may impair brain energetic and mitochondrial integrity and hence the ability of neuronal cells to cope with oxidative stress or mitochondrial toxins.

5. CONCLUSIONS

In this study, the intrastriatal injection of LPS in rats produced an increased oxidative stress and neuroinflammation associated with marked neurodegeneration in a number of brain areas. Cannabis treatment was able to decrease oxidative stress but failed to provide neuroprotection.

Author contribution

OMEAS and SAA designed the study and conducted the research and analysis. EAO performed the histopathology and its interpretation. YER performed biochemical studies. OMEAS performed the interpretation of data, wrote and prepared the manuscript. OMEAS, SAA, YER and EAO approved the final version of the manuscript.

Informed consent

Not applicable.

Ethical approval

The experimental procedures were performed in compliance with the institutional Ethics Committee and with the Guide for Care and Use of Laboratory Animals of US National Institutes of Health (Publication No. 85-23, revised 1996).

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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